Exhibit B

1: Lancet. 1997 Apr 19;349(9059):1137-41. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

BCL-2 antisense therapy in patients with non-Hodgkin lymphoma.

Webb A, Cunningham D, Cotter F, Clarke PA, di Stefano F, Ross P, Corbo M, Dziewanowska Z.

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BACKGROUND: Overexpression of BCL-2 is common in non-Hodgkin lymphoma and leads to resistance to programmed cell death (apoptosis) and promotes tumorigenesis. Antisense oligonucleotides targeted at the open reading frame of the BCL-2 mRNA cause a specific down-regulation of BCL-2 expression which leads to increased apoptosis. Lymphoma grown in laboratory animals responds to BCL-2 antisense oligonucleotides with few toxic effects. We report the first study of BCL-2 antisense therapy in human beings. METHODS: A daily subcutaneous infusion of 18-base, fully phosporothioated antisense oligonucleotide was administered for 2 weeks to nine patients who had BCL-2-positive relapsed non-Hodgkin lymphoma. Toxicity was scored by the common toxicity criteria, and tumour response was assessed by computed tomography scan. Efficacy was also assessed by quantification of BCL-2 expression; BCL-2 protein levels were measured by flow cytometry in samples from patients. FINDINGS: During the course of the study, the daily dose of BCL-2 antisense was increased incrementally from 4.6 mg/m2 to 73.6 mg/m2. No treatment-related toxic effects occurred, apart from local inflammation at the infusion site. In two patients, computed tomography scans showed a reduction in tumour size (one minor, one complete response). In two patients, the number of circulating lymphoma cells decreased during treatment. In four patients, serum concentrations of lactate dehydrogenase fell, and in two of these patients symptoms improved. We were able to measure BCL-2 levels by flow cytometry in the samples of five patients, two of whom had reduced levels of BCL-2 protein. INTERPRETATION: In patients with relapsing non-Hodgkin lymphoma, BCL-2 antisense therapy led to an improvement in symptoms, objective biochemical and radiological evidence of tumour response, and down-regulation of the BCL-2 protein in some patients. Our findings are encouraging and warrant further investigations of BCL-2 antisense therapy in cancer treatment.

Publication Types:

- Clinical Trial
- Clinical Trial, Phase I

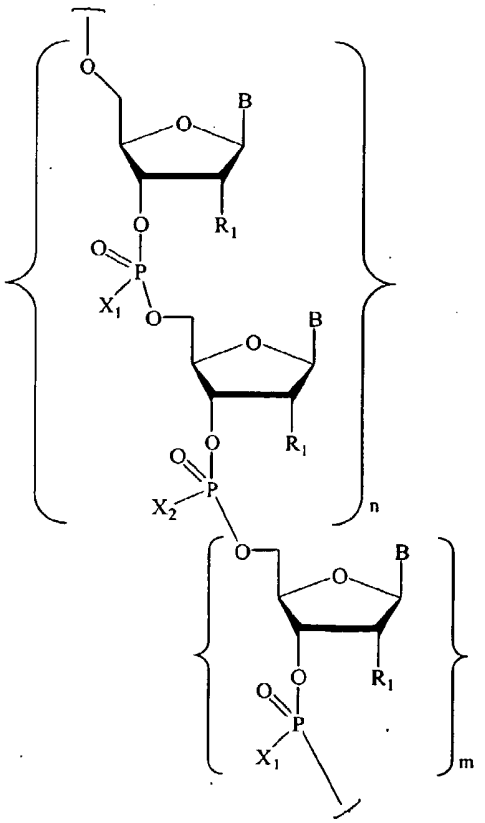
PMID: 9113013 [PubMed - indexed for MEDLINE]

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1-27. (Canceled).

28. (Currently amended) A method ~~of treating an organism having a disease characterized by the undesired production of a protein, said method~~ comprising contacting ~~said~~ an organism with a compound of formula:



wherein:

each B is a nucleobase;

one of X_1 or X_2 is O, and the other of X_1 or X_2 is S;

each R_1, is, independently, H, hydroxyl, C_1-C_{20} alkyl, C_3-C_{20} alkenyl, C_2-C_{20} alkynyl, halogen, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-

aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or R_1 is a group of formula $Z\text{-}R_{22}\text{-}(R_{23})_v$;

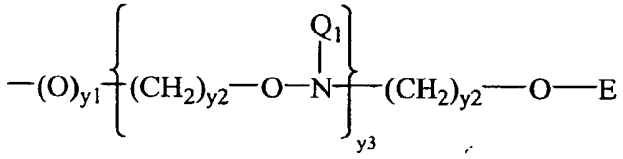
Z is O, S, NH, or $N\text{-}R_{22}\text{-}(R_{23})_v$;

R_{22} is $C_1\text{-}C_{20}$ alkyl, $C_2\text{-}C_{20}$ alkenyl, or $C_2\text{-}C_{20}$ alkynyl;

R_{23} is hydrogen, amino, halogen, hydroxyl, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

v is from 0 to about 10;

or R_1 has the formula:



wherein:

y1 is 0 or 1;

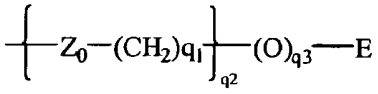
y2 is independently 0 to 10;

y3 is 1 to 10;

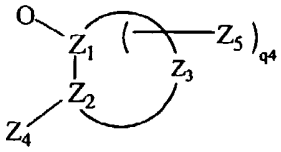
E is $C_1\text{-}C_{10}$ alkyl, $N(Q_1)(Q_2)$ or $N{=}C(Q_1)(Q_2)$;

each Q_1 and Q_2 is, independently, H, $C_1\text{-}C_{10}$ alkyl, substituted alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support; or Q_1 and Q_2, together, are joined in a nitrogen protecting group or a ring structure that can include at least one additional heteroatom selected from N and O;

or R_1 has one of formula I or II:



I



II

wherein:

Z_0 is O, S, or NH;

q^1 is from 0 to 10;

q^2 is from 1 to 10;

q^3 is 0 or 1;

q^4 is, 0, 1 or 2;

Z_4 is OM_1, SM_1, or $N(M_1)_2$;

each M_1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)M_2$, $C(=O)N(H)M_2$ or $OC(=O)N(H)M_2$;

M_2 is H or C_1-C_8 alkyl;

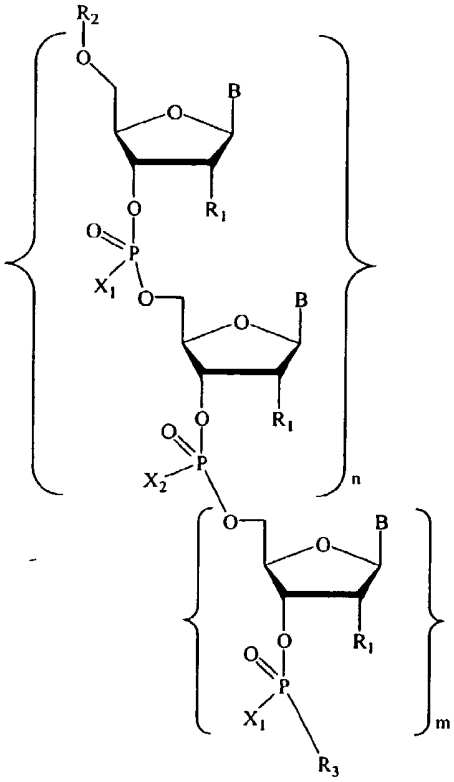
Z_1, Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms, or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur, and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic; and

Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, $N(Q_1)(Q_2)$, OQ_1, halo, SQ_1 or CN;

n is from 2 to 50; and

m is 0 or 1.

29. (Currently amended) A method ~~of treating an organism having a disease characterized by the undesired production of a protein, said method~~ comprising contacting ~~said~~ an organism with a compound of formula:



wherein:

each B is a nucleobase;

X_1 is S;

X_2 is O;

each R_1, is, independently, H, hydroxyl, C_1-C_{20} alkyl, C_3-C_{20} alkenyl, C_2-C_{20} alkynyl, halogen, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or R_1 is a group of formula $Z\text{-}R_{22}\text{-}(R_{23})_v$;

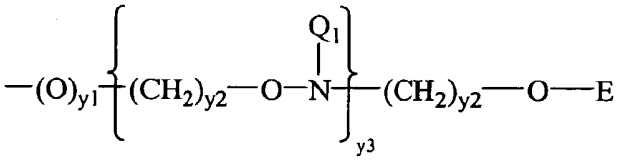
Z is O, S, NH, or $N\text{-}R_{22}\text{-}(R_{23})_v$;

R_{22} is C_1-C_{20} alkyl, C_2-C_{20} alkenyl, or C_2-C_{20} alkynyl;

R_{23} is hydrogen, amino, halogen, hydroxyl, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

v is from 0 to about 10;

or R_1 has the formula:



y1 is 0 or 1;

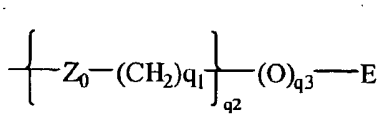
y2 is independently 0 to 10;

y3 is 1 to 10;

E is C_1-C_{10} alkyl, $N(Q_1)(Q_2)$ or $N{=}C(Q_1)(Q_2)$;

each Q_1 and Q_2 is, independently, H, C_1-C_{10} alkyl, substituted alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support; or Q_1 and Q_2, together, are joined in a nitrogen protecting group or a ring structure that can include at least one additional heteroatom selected from N and O;

or R_1 has one of formula I or II:



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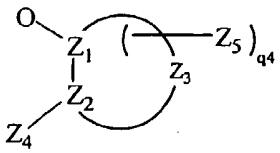


II

wherein:

Z_0 is O, S, or NH;

q^1 is from 0 to 10;

q^2 is from 1 to 10;

q^3 is 0 or 1;

q^4 is, 0, 1 or 2;

Z_4 is OM_1, SM_1, or $N(M_1)_2$;

each M_1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)M_2$, $C(=O)N(H)M_2$ or $OC(=O)N(H)M_2$;

M_2 is H or C_1-C_8 alkyl;

Z_1, Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms, or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur, and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic; and

Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, $N(Q_1)(Q_2)$, OQ_1, halo, SQ_1 or CN;

n is from 2 to 50; and

m is 0 or 1;

R_2 is H, a hydroxyl protecting group, or an oligonucleotide; and

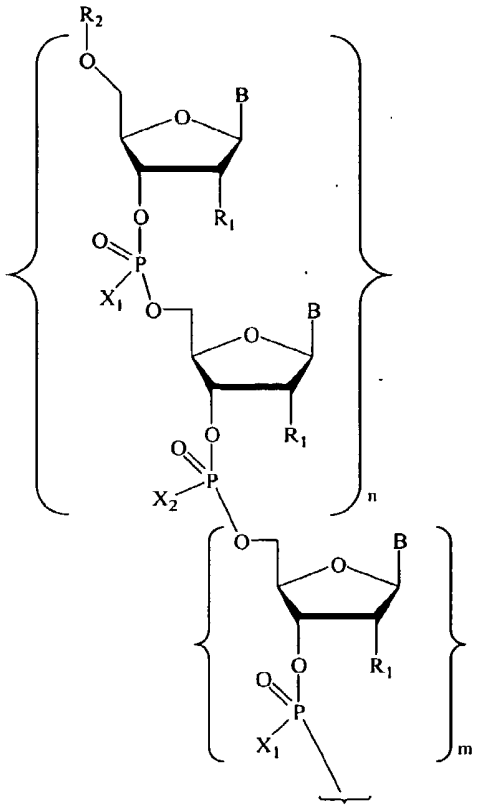
R_3 is OH, an oligonucleotide, or a linker connected to a solid support.

30. (Currently amended) A method ~~of treating an organism having a disease characterized by the undesired production of a protein, said method~~ comprising contacting ~~said~~ an organism with a compound of formula:

$$(5')\ W^1\text{-}W^2\text{-}W^3\ (3')$$

wherein:

W^1 has the Formula:



wherein:

each B is a nucleobase;

one of X_1 or X_2 is O, and the other of X_1 or X_2 is S;

each R_1, is, independently, H, hydroxyl, C_1-C_{20} alkyl, C_3-C_{20} alkenyl, C_2-C_{20} alkynyl, halogen, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or R_1 is a group of formula Z-R_{22}-$(R_{23})_v$;

Z is O, S, NH, or N-R_{22}-$(R_{23})_v$;

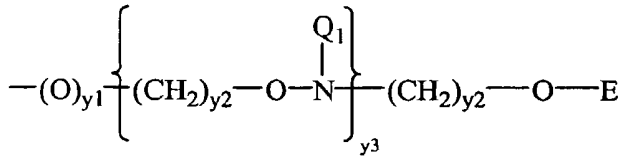
R_{22} is C_1-C_{20} alkyl, C_2-C_{20} alkenyl, or C_2-C_{20} alkynyl;

R_{23} is hydrogen, amino, halogen, hydroxyl, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, S-alkyl, NH-alkyl, N-dialkyl, O-

aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, amino, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

v is from 0 to about 10;

or R_1 has the formula:



y1 is 0 or 1;

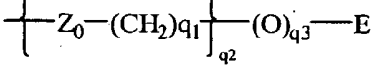
y2 is independently 0 to 10;

y3 is 1 to 10;

E is C_1-C_{10} alkyl, $N(Q_1)(Q_2)$ or $N{=}C(Q_1)(Q_2)$;

each Q_1 and Q_2 is, independently, H, C_1-C_{10} alkyl, substituted alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support; or Q_1 and Q_2, together, are joined in a nitrogen protecting group or a ring structure that can include at least one additional heteroatom selected from N and O;

or R_1 has one of formula I or II:



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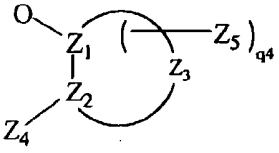


II

wherein:

Z_0 is O, S, or NH;

q^1 is from 0 to 10;

q^2 is from 1 to 10;

q^3 is 0 or 1;

q^4 is, 0, 1 or 2;

Z_4 is OM_1, SM_1, or $N(M_1)_2$;

each M_1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)M_2$, $C(=O)N(H)M_2$ or $OC(=O)N(H)M_2$;

M_2 is H or C_1-C_8 alkyl;

Z_1, Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms, or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur, and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic; and

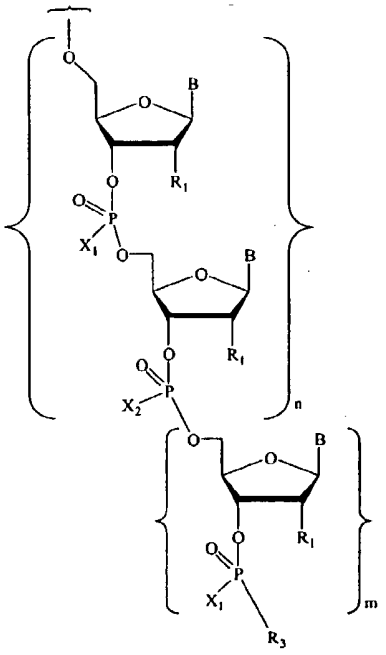
Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, $N(Q_1)(Q_2)$, OQ_1, halo, SQ_1 or CN;

n is from 2 to 50; and

m is 0 or 1;

R_2 is H, a hydroxyl protecting group, or an oligonucleotide;

W^3 has the Formula:



wherein R_3 is OH, an oligonucleotide, or a linker connected to a solid support; and

W^2 is a plurality of covalently bound nucleosides linked by phosphodiester or phosphorothioate linkages.

31-51. (Canceled).

52. (Previously presented) The method of claim 28 wherein R_1 is -O-CH_2-CH_2-O-CH_3.

53. (Previously presented) The method of claim 28 wherein n is about 5 to about 50.

54. (Previously presented) The method of claim 28 wherein n is about 8 to about 30.

55. (Previously presented) The method of claim 28 wherein n is about 4 to about 15.

56. (Previously presented) The method of claim 28 wherein n is 2 to about 10.

57. (Previously presented) The method of claim 29 wherein R_1 is -O-CH_2-CH_2-O-CH_3.

58. (Previously presented) The method of claim 29 wherein R_2 is H, and R_3 is OH.

59. (Previously presented) The method of claim 29 wherein R_2 is a phosphodiester-linked oligonucleotide or a phosphorothioate linked oligonucleotide.

60. (Previously presented) The method of claim 29 wherein R_3 is a phosphodiester-linked oligonucleotide or a phosphorothioate linked oligonucleotide.

61. (Previously presented) The method of claim 29 R_2 and R_3 are each a phosphodiester-linked oligonucleotide or a phosphorothioate linked oligonucleotide.

62. (Previously presented) The method of claim 30 wherein R_1 is $-O-CH_2-CH_2-O-CH_3$.

63. (Previously presented) The method of claim 30 wherein R_2 is H, and R_3 is OH.

64. (Previously presented) The method of claim 30 wherein n is about 5 to about 50.

65. (Previously presented) The method of claim 30 wherein n is about 8 to about 30.

66. (Previously presented) The method of claim 30 wherein n is about 4 to about 15.

67. (Previously presented) The method of claim 30 wherein n is 2 to about 10.

68. (Previously presented) The method of claim 30 wherein W^2 is a plurality of covalently bound nucleosides linked by phosphodiester linkages.

69. (Previously presented) The method of claim 30 wherein W^2 is a plurality of covalently bound nucleosides linked by phosphorothioate linkages.